Transgenic Res (2007) 16:581–585
DOI 10.1007/s11248-007-9111-1

PERSPECTIVE

Metabolic engineering of carotenoid accumulation by creating a metabolic sink

Li Li · Joyce Van Eck

Received: 14 May 2007 / Accepted: 24 May 2007 / Published online: 6 July 2007

Abstract Carotenoids are highly beneficial for human nutrition and health because they provide essential nutrients and important antioxidants in our diets. However, many food crops, especially the major staple crops contain only trace to low amounts of carotenoids. Although significant progress has been made in developing food crops rich in carotenoids by altering the expression of carotenoid biosynthetic genes, in many cases it has proved to be difficult to reach the desired levels of carotenoid enrichment. The recent identification and characterization of a novel gene mutation in cauliflower reveals that creating a metabolic sink to sequester carotenoids is an important mechanism to control carotenoid accumulation in plants. The successful demonstration of increased carotenoid accumulation in association with the formation of sink structures in transgenic crops offers a new and alternative approach to increase carotenoid content. Manipulation of the formation of metabolic sink along with the catalytic activity of the pathway may represent a promising strategy for maximally improving the nutritional quality of food crops.

L. Li (✉)
USDA-ARS, Plant, Soil and Nutrition Laboratory,
Cornell University, Ithaca, NY 14853, USA
e-mail: ll37@cornell.edu

J. Van Eck
Boyce Thompson Institute for Plant Research,
Cornell University, Ithaca, NY 14853, USA

Keywords Carotenoids · Metabolic sink · Carotenoid sequestering structures · Chromoplasts · Cauliflower *Or* gene

Introduction

Carotenoids represent a class of red, orange and yellow pigments widely distributed in nature. They are mainly C_{40} isoprenoids and play a critical role in human nutrition and health. Because humans are unable to synthesize vitamin A *de novo* from endogenous isoprenoids precursors, plant carotenoids provide the primary dietary source of provitamin A. The deficiency of vitamin A, which remains one of the most noticeable nutritional problems in many parts of the world, affects an estimated 250 million preschool children and causes blindness in up to 500,000 of them annually (World Health Organization). Apart from the nutritional significance, carotenoids as antioxidants have been implicated in reducing the risk of cancer and cardiovascular diseases (Giovannucci 1999; Hadley et al. 2002). Some carotenoids also offer protection against age-related eye diseases, such as macular degeneration, the leading cause of blindness in the elderly (Krinsky et al. 2003). Thus, development of carotenoid-enriched food crops provides the most effective and sustainable approach to maximize the nutritional and health benefits of carotenoids to a large number of population in the world.

Transgenic approaches to alter the expression of carotenoid biosynthetic genes have proved to be successful in engineering of carotenoid content in some food crops for nutritional enhancement (Fraser and Bramley 2004; Taylor and Ramsay 2005; Botella-Pavia and Rodriguez-Concepcion 2006; Sandmann et al. 2006). The best known example is Golden Rice (Ye et al. 2000; Beyer et al. 2002). Through tissue-specific overexpression of several carotenoid biosynthetic genes, the rice endosperm has been engineered to contain up to 31 μg g^{-1} β-carotene, a level adequate to provide the recommended dietary allowance of provitamin A for children in an average daily consumption of rice (Paine et al. 2005). Recently, a similar approach has been employed in successfully producing "golden" potato tubers (Diretto et al. 2007). Also, seed-specific overexpression of a key gene in carotenoid biosynthetic pathway produces "golden" canola seeds containing up to a 50-fold increase in total carotenoids (Shewmaker et al. 1999). In addition, metabolic engineering of potato and tomato has led to the production of more β-carotene, lycopene, and zeaxanthin (Romer et al. 2000; Fraser et al. 2002; Ducreux et al. 2005), and the accumulation of astaxanthin, a new and high-economic value carotenoid, in potato tubers (Gerjets and Sandmann 2006). In spite of these achievements, in many cases manipulation of the catalytic genes alone seems to be insufficient to achieve the desired levels of carotenoid enhancement in food crops (Fraser and Bramley 2004; Wurtzel 2004).

Carotenoids in plants are synthesized in the membranes of nearly all types of plastids and accumulate in high levels in chromoplasts of many flowers, fruits, and roots (Howitt and Pogson 2006). Chromoplasts possess a unique mechanism to accumulate massive amounts of carotenoids by generating novel carotenoid-lipoprotein substructures inside chromoplasts. These structures are referred as carotenoid sequestering structures (Bartley and Scolnik 1995; Vishnevetsky et al. 1999). Such structures serve as deposition sinks to sequester excess carotenoids and may also prevent the end products of the carotenoid biosynthetic pathway from overloading the site of carotenoid biosynthesis in chromoplast membranes (Deruere et al. 1994; Rabbani et al. 1998; Al Babili et al. 1999). Thus, control of the formation of a metabolic sink can offer a novel and complement approach to mediate carotenoid accumulation in food crops.

Cauliflower *Orange* (*Or*) gene and carotenoid accumulation

Direct evidence that strongly supports this notion comes from our work on isolation and functional characterization of a novel carotenoid gene mutation in cauliflower (Lu et al. 2006). This *Or* gene, which encodes a DnaJ cysteine-rich domain-containing protein, confers orange curd with high levels of β-carotene accumulation (Fig. 1A). Rather than directly regulating carotenoid biosynthesis, the *Or* gene appears to mediate the differentiation of proplastids and/or non-colored plastids in apical shoot and inflorescence meristematic tissues of curd into chromoplasts for the associated carotenoid accumulation (Li et al. 2001; Lu et al. 2006). Transformation of the *Or* gene into wild type cauliflower converts the white color of curd tissue into distinct orange color with increased levels of β-carotene. Examination of the cytological effects of the *Or* transgene revealed that expression of the *Or* transgene leads to the formation of large membranous chromoplasts in the cauliflower curd cells of the *Or* transformants (Lu et al. 2006).

When the *Or* gene under the control of a potato granule-bound starch synthase (GBSS) promoter was introduced into a major staple crop, potato, expression of the *Or* gene in the transgenic potato tubers resulted in the production of orange-yellow flesh tubers (Fig. 1B). The total carotenoid levels in the *Or* transgenic lines were up to sixfold higher than the non-transformed and vector-only controls. Further examination of the cellular contents of these transgenic tubers by light microscopy showed that while the tubers in the non-transformed and vector-only controls contain exclusively various sizes of starch grains in amyloplasts, the *Or* transgenic tubers have additional orange bodies. These orange bodies include intact chromoplasts and a large number of more sharply outlined orange structures of helical sheets and fragments released from chromoplasts (manuscript submitted). In comparison, examination of potato tubers from a high carotenoid breeding line 91E22 (Brown et al. 2005) revealed that high levels of carotenoid accumulation per se would not lead to the formation of carotenoid-sequestering structures in the tubers (Lopez and Li, unpublished data). Thus, the *Or* gene-associated carotenoid accumulation in these transgenic tubers is most likely due to the formation of carotenoid sequestering structures in

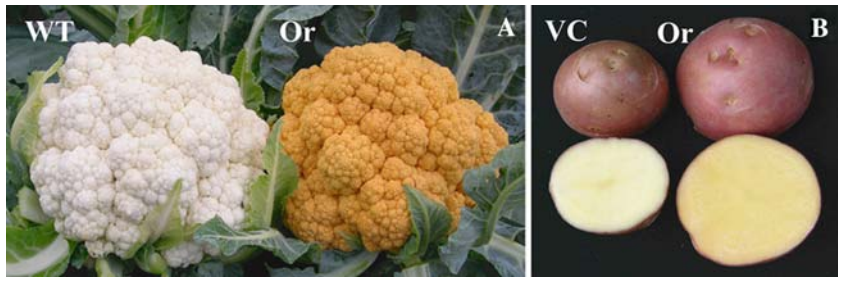

Fig. 1 Phenotype of cauliflower curds and potato tubers in the presence and absence of the *Or* gene. A. Curd of wild type (WT) and commercial hybrid orange cauliflower (Or). B. Transgenic potato tubers of vector-only control (VC) and *Or* transformant (Or). The color is associated with accumulation of carotenoids

chromoplasts, which provide a metabolic sink to facilitate accumulation of carotenoids.

Synthesis of carotenoid sequestering structures and carotenoid accumulation in low-pigmented tissues of crops

Carotenoid sequestering structures are composed of carotenoids, lipid, and proteins. Variation of these components attributes to different types of carotenoid accumulation structures in chromoplasts (Camara et al. 1995; Vishnevetsky et al. 1999). The biosynthesis of components of the carotenoid sequestering structures has been shown to play a fundamental role in carotenoid sequestration and accumulation. For example, in red pepper and cucumber flowers, carotenoids accumulate in specific lipoprotein fibrils in chromoplasts, and the massive synthesis of carotenoids during flower development and fruit ripening is parallel to the accumulation of a carotenoid-associated protein, fibrillin or CHRC (Deruere et al. 1994; Vishnevetsky et al. 1996). In daffodil flowers, high levels of carotenoid accumulation are concomitant with the massive proliferation of chromoplast internal membranes in providing a lipophilic sink (Al Babili et al. 1999). Also, cells of the unicellular alga *Dunaliiella bardawil* overproduce β-carotene in plastids under stress. Such an overproduction was found to depend on the formation of the lipophilic sequestering structures rather than up-regulating the expression of carotenoid genes or enzymes (Rabbani et al. 1998). Further, carotenoid accumulation in the tomato *high pigment-1* mutant is associated with an increased plastid number and size for deposition (Cookson et al. 2003; Liu et al. 2004). These studies clearly show that the formation of carotenoid sequestering structures for deposition plays an important role in regulating carotenoid accumulation.

Manipulation of the formation of deposition sinks offers a new strategy for metabolic engineering of carotenoid content in storage tissues of food crops. In many white and low-pigmented tissues of roots and seeds, low levels of carotenoids accumulate in plastids such as in amyloplasts of starch-storing seeds of wheat, rice, barley, and maize, and in elaioplasts of lipid-storing seeds of canola, sunflower, and pumpkin (Howitt and Pogson 2006). In spite of low amounts of carotenoid accumulation, many carotenoid genes involved in carotenoid biosynthesis are expressed in these tissues (Schaub et al. 2005; Diretto et al. 2006). Indeed, in some instances the carotenoid genes in the white tissues are expressed at significant levels, despite trace or low amounts of carotenoids accumulated (Li et al. 2001). The apparent lack of high levels of carotenoid accumulation in those white or low-pigmented tissues of crops could be due to low metabolic flux into the carotenoid biosynthetic pathway, or inactivation and absence of a key gene in the biosynthetic pathway such as in the cases of rice endosperm (Schaub et al. 2005) and white maize kernel (Wurtzel 2004). It could also be due to lack of a deposition sink to efficiently sequester the end products of carotenoid biosynthetic pathway. For example, the white cauliflower curd appears to have the capacity to synthesize carotenoids but contains negligible amounts of carotenoids (Li et al. 2001). The presence of the likely gain-of-function mutation of the *Or* gene induces the formation of sequestering structures in chromoplasts, resulting in dramatically accumulation of β-carotene without alteration of the expression of carotenoid

biosynthetic genes. This demonstrates that creating a metabolic sink has a profound effect on carotenoid accumulation in the low-pigmented tissues of food crops.

It should be noted that enhancing sink capacity for associated carotenoid accumulation in storage tissues of crops requires the presence of all functional genes and enzymes in the biosynthetic pathway. The extent of carotenoid enhancement depends on the maximal potential catalytic activity of this pathway in particular tissues of crops. Also, the specific carotenoids accumulated depend on the endogenous rate-limiting steps of the pathway in the tissues of crops. When the enhanced sink capacity provides a pulling force to draw the metabolic flux through the carotenoid biosynthetic pathway, the limiting catalytic activities of rate-limiting steps will result in the accumulation of the immediate precursors in specific tissues of crops.

Although it has been demonstrated that modification of sink capacity provides a new strategy to enhance carotenoids in storage tissues of food crops, the concomitant manipulation of catalytic activity with the capacity of sequestering carotenoids may be a more effective strategy to quantitatively and qualitatively modify carotenoids. Previous studies have shown that overexpression of genes in the carotenoid biosynthetic pathway results in the production of food crops with increased levels of carotenoids (reviewed by Fraser and Bramley 2004; Taylor and Ramsay 2005). This approach is particularly effective with upregulation of the potential rate-limiting steps (Shewmaker et al. 1999), using the genes encoding enzymes with significant high enzymatic activities (Paine et al. 2005), and a combination of expressing multiple genes in the pathway (Diretto et al. 2007). By providing deposition sinks to effectively sequester carotenoids, it is possible to further increase carotenoid content in many food crops to meet the requirement for optimal human nutrition and health.

Conclusions

Manipulation of carotenoid content in food crops has been primarily focused on engineering of catalytic activity of this pathway by altering the expression of carotenoid biosynthetic genes. In some cases, this is not sufficient to enhance carotenoid contents to levels required for optimal human nutrition and health. A new and alternative approach to enhance carotenoid levels in food crops is based on regulating the formation of metabolic sinks to effectively sequester carotenoids. Such sinks exert their positive effect on carotenoid accumulation by pulling the pathway toward completion. This approach, in combination with manipulation of catalytic activity of the pathway, may prove to be an effective and efficient strategy to dramatically enrich carotenoid content in low-pigmented tissues of food crops.

Acknowledgements We thank X. Zhou for the photograph. We are grateful to many colleagues for their contribution to this work. This research was supported by USDA National Research Initiative Grants, ARS Headquarter Postdoctoral Research Associated Fund, the Triad Foundation, and Helen Graham Charitable Foundation.

References

Al Babili S, Hartung W, Kleinig H, Beyer P (1999) CPTA modulates levels of carotenogenic proteins and their mRNAs and affects carotenoid and ABA content as well as chromoplast structure in *Narcissus pseudonarcissus* flowers. Plant Biol 1:607–612

Bartley GE, Scolnik PA (1995) Plant carotenoids: pigments for photoprotection, visual attraction, and human health. Plant Cell 7:1027–1038

Beyer P, Al Babili S, Ye X, Lucca P, Schaub P, Welsch R, Potrykus I (2002) Golden Rice: Introducing the beta-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. J Nutr 132:506S–510S

Botella-Pavia P, Rodriguez-Concepcion M (2006) Carotenoid biotechnology in plants for nutritionally improved foods. Physiol Plant 126:369–381

Brown CR, Culley D, Yang CP, Durst R, Wrolstad R (2005) Variation of anthocyanin and carotenoid contents and associated antioxidant values in potato breeding lines. J Am Soc Hortic Sci 130:174–180

Camara B, Hugueney P, Bouvier F, Kuntz M, Moneger R (1995) Biochemistry and molecular biology of chromoplast development. Int Rev Cytol 163:175–247

Cookson PJ, Kiano JW, Shipton CA, Fraser PD, Romer S, Schuch W, Bramley P, Pyke KA (2003) Increases in cell elongation, plastid compartment size and phytoene synthase activity underlie the phenotype of the *high pigment-1* mutant of tomato. Planta 217:896–903

Deruere J, Romer S, d'Harlingue A, Backhaus RA, Kuntz M, Camara B (1994) Fibril assembly and carotenoid overaccumulation in chromoplasts: a model for supramolecular lipoprotein structures. Plant Cell 6:119–133

Diretto G, Al-Babili S, Tavazza R, Papacchioli V, Beyer P, Giuliano G (2007) Metabolic engineering of potato carotenoid content through tuber-specific overexpression of a bacterial mini-pathway. PLoS ONE 2:e350

Diretto G, Tavazza R, Welsch R, Pizzichini D, Mourgues F, Papacchioli V, Beyer P, Giuliano G (2006) Metabolic engineering of potato tuber carotenoids through tuber-specific silencing of lycopene epsilon cyclase. BMC Plant Biol 6:13

Ducreux LJ, Morris WL, Hedley PE, Shepherd T, Davies HV, Millam S, Taylor MA (2005) Metabolic engineering of high carotenoid potato tubers containing enhanced levels of beta-carotene and lutein. J Exp Bot 56:81–89

Fraser PD, Bramley PM (2004) The biosynthesis and nutritional uses of carotenoids. Prog Lipid Res 43:228–265

Fraser PD, Romer S, Shipton CA, Mills PB, Kiano JW, Misawa N, Drake RG, Schuch W, Bramley PM (2002) Evaluation of transgenic tomato plants expressing an additional phytoene synthase in a fruit-specific manner. Proc Natl Acad Sci USA 99:1092–1097

Gerjets T, Sandmann G (2006) Ketocarotenoid formation in transgenic potato. J Exp Bot 57:3639–3645

Giovannucci E (1999) Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. J Natl Cancer Inst 91:317–331

Hadley CW, Miller EC, Schwartz SJ, Clinton SK (2002) Tomatoes, lycopene, and prostate cancer: progress and promise. Exp Biol Med 227:869–880

Howitt CA, Pogson BJ (2006) Carotenoid accumulation and function in seeds and non-green tissues. Plant Cell Environ 29:435–445

Krinsky NI, Landrum JT, Bone RA (2003) Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. Annu Rev Nutr 23:171–201

Li L, Paolillo DJ, Parthasarathy MV, Dimuzio EM, Garvin DF (2001) A novel gene mutation that confers abnormal patterns of beta-carotene accumulation in cauliflower (*Brassica oleracea var. botrytis*). Plant J 26:59–67

Liu Y, Roof S, Ye Z, Barry C, van Tuinen A, Vrebalov J, Bowler C, Giovannoni J (2004) Manipulation of light signal transduction as a means of modifying fruit nutritional quality in tomato. Proc Natl Acad Sci USA 101:9897–9902

Lu S, Eck Van J, Zhou X, Lopez AB, O'Halloran DM, Cosman KM, Conlin BJ, Paolillo DJ, Garvin DF, Vrebalov J, Kochian LV, Kupper H, Earle ED, Cao J, Li L (2006) The cauliflower *Or* gene encodes a DnaJ cysteine-rich domain-containing protein that mediates high levels of beta-carotene accumulation. Plant Cell 18:3594–3605

Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, Wright SY, Hinchliffe E, Adams JL, Silverstone AL, Drake R (2005) Improving the nutritional value of Golden Rice through increased pro-vitamin A content. Nat Biotechnol 23:482–487

Rabbani S, Beyer P, Lintig J, Hugueney P, Kleinig H (1998) Induced beta-carotene synthesis driven by triacylglycerol deposition in the unicellular alga *Dunaliella bardawil*. Plant Physiol 116:1239–1248

Romer S, Fraser PD, Kiano JW, Shipton CA, Misawa N, Schuch W, Bramley PM (2000) Elevation of the provitamin A content of transgenic tomato plants. Nat Biotechnol 18:666–669

Sandmann G, Romer S, Fraser PD (2006) Understanding carotenoid metabolism as a necessity for genetic engineering of crop plants. Metab Eng 8:291–302

Schaub P, Al-Babili S, Drake R, Beyer P (2005) Why is golden rice golden (yellow) instead of red? Plant Physiol 138:441–450

Shewmaker CK, Sheehy JA, Daley M, Colburn S, Ke DY (1999) Seed-specific overexpression of phytoene synthase: increase in carotenoids and other metabolic effects. Plant J 20:401–412X

Taylor M, Ramsay G (2005) Carotenoid biosynthesis in plant storage organs: recent advances and prospects for improving plant food quality. Physiol Plant 124:143–151

Vishnevetsky M, Ovadis M, Vainstein A (1999) Carotenoid sequestration in plants: the role of carotenoid-associated proteins. Trends Plant Sci 4:232–235

Vishnevetsky M, Ovadis M, Itzhaki H, Levy M, Libal WY, Adam Z, Vainstein A (1996) Molecular cloning of a carotenoid-associated protein from *Cucumis sativus* corollas: homologous genes involved in carotenoid sequestration in chromoplasts. Plant J 10:1111–1118

Wurtzel ET (2004) Genomics, genetics, and biochemistry of maize carotneoid biosynthesis. In: Romeo J (ed) Recent Advances in Phytochemistry, vol 38. Elsevier Ltd., pp 85–110

Ye X, Al-Babili S, Kloti A, Zhang J, Lucca P, Beyer P, Potrykus I (2000) Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287:303–305